CompCytogen 15(4): 459–465 (2021)
doi: 10.3897/compcytogen.v15.i4.71295
https://compcytogen.pensoft.net

SHORT COMMUNICATION

COMPARATIVE Cytogenetics
A peer-reviewed open-access journal
International Journal of Plant & Animal Cytogenetics, Karyosystematics, and Molecular Systematics

A rare chromosomal polymorphism in a Kangayam bull (*Bos indicus*) of south India

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Academic editor: Nina Bulatova | Received 11 July 2021 | Accepted 11 October 2021 | Published 15 December 2021

http://zoobank.org/55232ED5-A017-456D-B118-13E7CB9E62DC

Citation: Harshini V, Kumarasamy P, Karthickeyan SMK (2021) A rare chromosomal polymorphism in a Kangayam bull (*Bos indicus*) of south India CompCytogen 15(4): 459–465. https://doi.org/10.3897/compcytogen.v15.i4.71295

Abstract

A chromosomal polymorphism was detected on karyological screening of Kangayam breeding sires prior to subjecting them for frozen semen collection. One bull possessed the chromosomal complement 2n = 60, consisting of 58 acrocentric autosomes, one large sub-metacentric X-chromosome, and one small acrocentric Y-chromosome with a small visible p-arm, which was further confirmed using CBG- and GTG-banding. This polymorphism was attributed to a heterochromatin variation of the acrocentric Y-chromosome routine in the *Bos indicus* Linnaeus, 1758 cattle.

Keywords

Heterochromatin variation, individual chromosomal polymorphism, karyological screening

Introduction

The Kangayam breed of cattle is a pride of Tamil Nadu and native to south India. It is well known for its excellent draught qualities, adaptation to poor nutrition and longevity (Kandasamy 2001). Despite large scale transformation and decline in agricultural practices, there is still a demand for the Kangayam cattle from the neighboring states such as Kerala, Karnataka and Andhra Pradesh. The breed has been transported to

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countries like Brazil, Malaysia, Philippines and Sri Lanka. Given its importance, the breed has been characterized phenotypically as well as through cytogenetic (Kumarasamy et al. 2006) and microsatellite analyses (Karthickeyan et al. 2009).

In the process of evolution, chromosomes have undergone rearrangements and form species-specific karyotypes. Iannuzzi and Di Meo (1995) stated that centric fusion translocations, and peri- or paracentric inversions along with the loss or gain of heterochromatin appeared to be the main chromosomal rearrangements occurred and thus differentiated the chromosomal complements across the bovid species. The variations in the size of the Y-chromosomes among subspecies are proportional to the amount of heterochromatin present (Cabelova et al. 2012). It shows the involvement of heterochromatin in the karyotype evolution of taxa of higher as well as lower ranks.

The chromosomal complements of *Bos indicus* Linnaeus, 1758 and *Bos taurus* Linnaeus, 1758 cattle are highly similar except for the Y-chromosome morphology being acrocentric (one arm) and sub-meta/metacentric (two arms), respectively. This morphological difference is due to the pericentric inversion which might have been occurred at the time of divergence (Goldammer et al. 1997; Di Meo et al. 2005). In the present study, a phenotypically healthy Kangayam bull was found carrying a different type of Y-chromosome on routine karyological screening, which was subjected to banding for unequivocal identification of chromosome morphology.

Material and methods

A total of 46 blood samples of Kangayam bulls were received for routine cytogenetic screening before using them for semen collection (14 from Buffalo Frozen Semen Station, 16 from Kangayam Cattle Research Station and 16 from field progeny tested animals in Tamil Nadu).

Metaphase chromosomes were obtained using short term lymphocyte culture technique (Moorhead et al. 1960), standardized with minor modifications in the Cytogenetics Laboratory of Department of Animal Genetics and Breeding, Madras Veterinary College, Chennai, India. The chromosome spreads were examined under Olympus microscope (BX61, USA) and more than 200 metaphases were screened. The good metaphase spreads were photographed using applied spectral imaging software. The cell fixation from one Kangayam bull carrying an unusual Y-chromosome was subsequently further studied using different cytogenetic banding techniques.

The chromosome preparations were CBG-banded using barium hydroxide [$Ba(OH)_2$] treatment as described by Sumner et al. (1972) with minor modifications (Harshini et al. 2020). GTG-banding technique was done as described by Seabright (1971) with modifications in concentration of the trypsin and exposure time. Slides aged for 5–7 days were immersed in Sorenson's phosphate buffer for 2–3 seconds and transferred to 0.025 per cent trypsin solution for 10–14 seconds. Then the slides were immediately transferred to Sorenson's phosphate buffer to stop the action of trypsin, washed twice in double distilled water and subsequently air-dried. The chromosomes

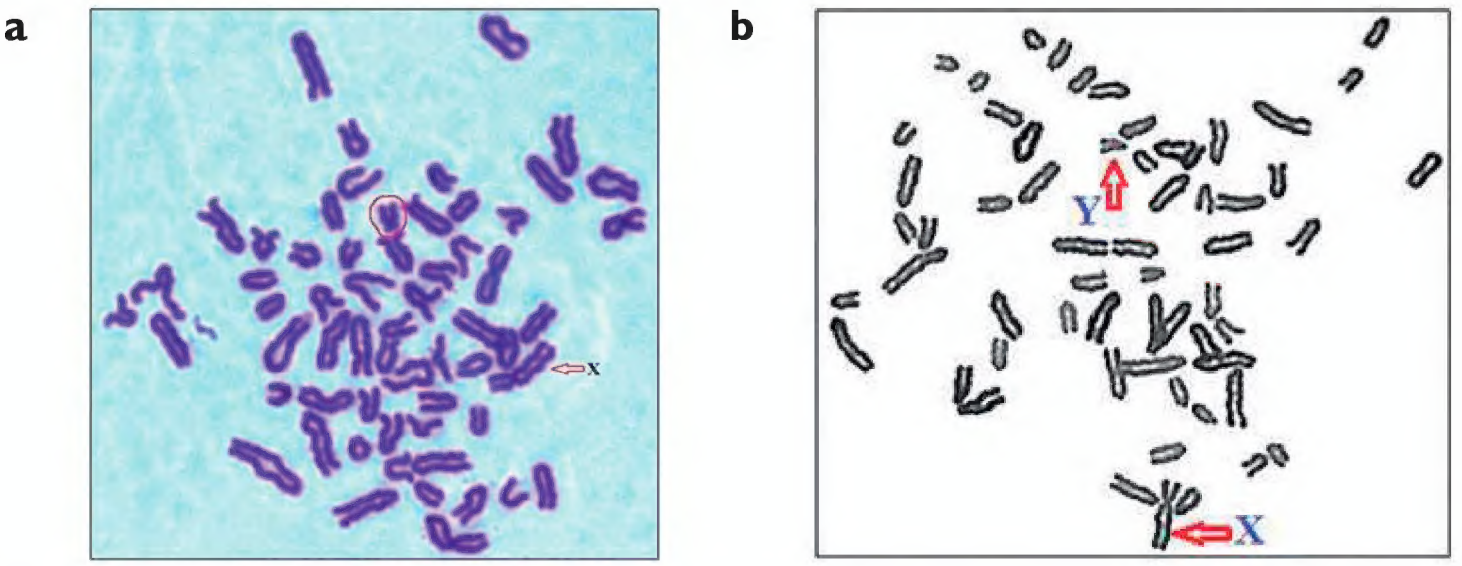

Figure 1. Giemsa-stained metaphase spreads of Kangayam bulls showing general similarity of acrocentric autosome set and X-chromosome, and different morphology of Y-chromosome: subacrocentric with small p-arm (**a**, encircled) and typical acrocentric (**b**).

were stained with 4 per cent Giemsa for 15 minutes and rinsed thoroughly in two consecutive washes in double distilled water. The chromosomes were then observed under microscope, photographed and karyotyped.

Results and discussion

All the Kangayam bulls, except the one investigated in detail, found to have 60 chromosomal diploid set with 29 pairs of acrocentric autosomes, one large sub-metacentric X- and the smallest acrocentric Y-chromosome. One bull showed similar chromosomal profile except for the presence of an unpaired smallest subacrocentric chromosome possessing a small p-arm (Fig. 1) in all the spreads screened. A similar kind of chromosomal complement was reported earlier by Iannuzzi et al. (2001) in Chainina cattle (*Bos taurus*) and considered as a sex-autosomal reciprocal translocation between the chromosomes Y and 9. Therefore, this Kangayam sample was further investigated using CBG- and GTG-banding techniques.

CBG-banding revealed that all the acrocentric chromosomes each showed characteristically a positive C-band as a distinctly-stained centromeric region. The X-chromosome was stained lightly across its entire length (Fig. 2). This is like the standard CBG-banding pattern of cattle as reported for Red Danish (Hansen 1973), *Bos taurus* L. (Iannuzzi and Berardino 1985), Jersey crossbreds (Chauhan et al. 2009), Tho-Tho cattle (Longkumer et al. 2015), mithun (Ezung 2016) and Nellore cattle (Amancio et al. 2019). However, the unpaired acrocentric chromosome with a small p-arm also stained darkly throughout the length showing no centromere banding (Fig. 2). Thus, it was confirmed as a sex chromosome (Y) and its small extra p-arm was not a translocated portion of any autosome which arms are lightly C-stained.

Upon GTG-banding, the Y-chromosome displayed a rearrangement in the distribution of G-bands divided for two arms (p, q) in the subacrocentric Y-chromo-

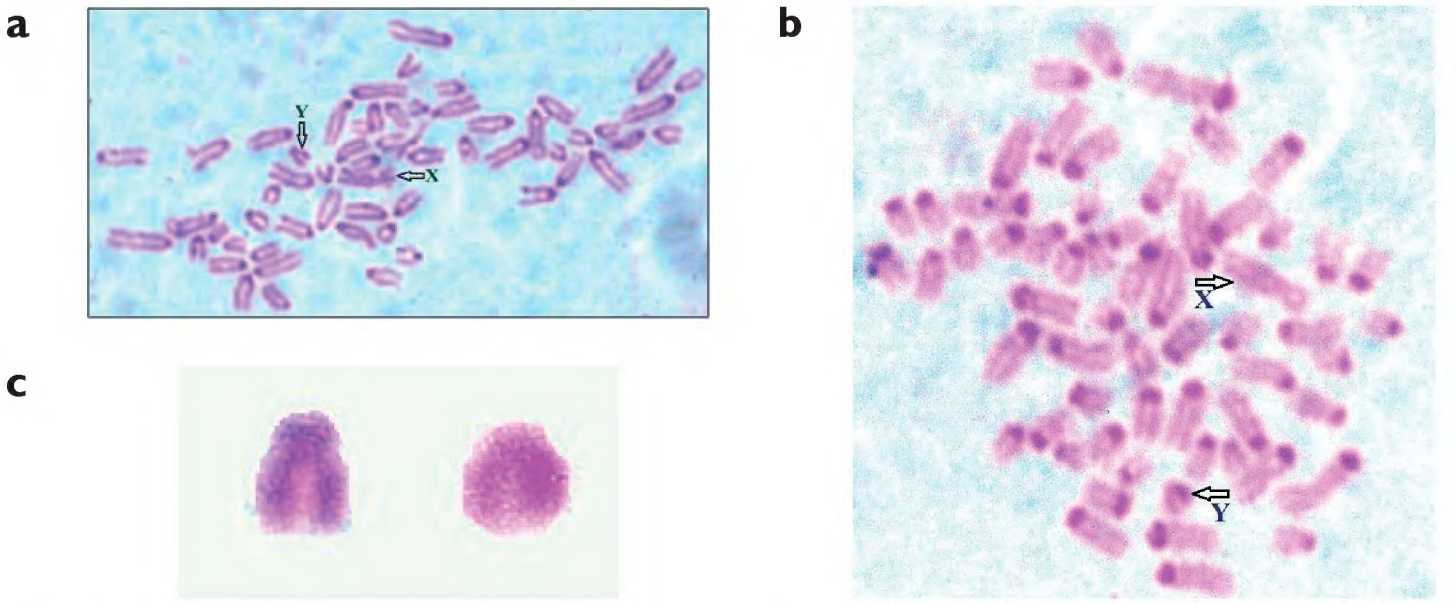

Figure 2. CBG-banded metaphase spreads of Kangayam bulls (**a, b**) bearing C-positive heterochromatic Y chromosome of two alternative types - subacrocentric (**c**, left) or acrocentric (**c**, right).

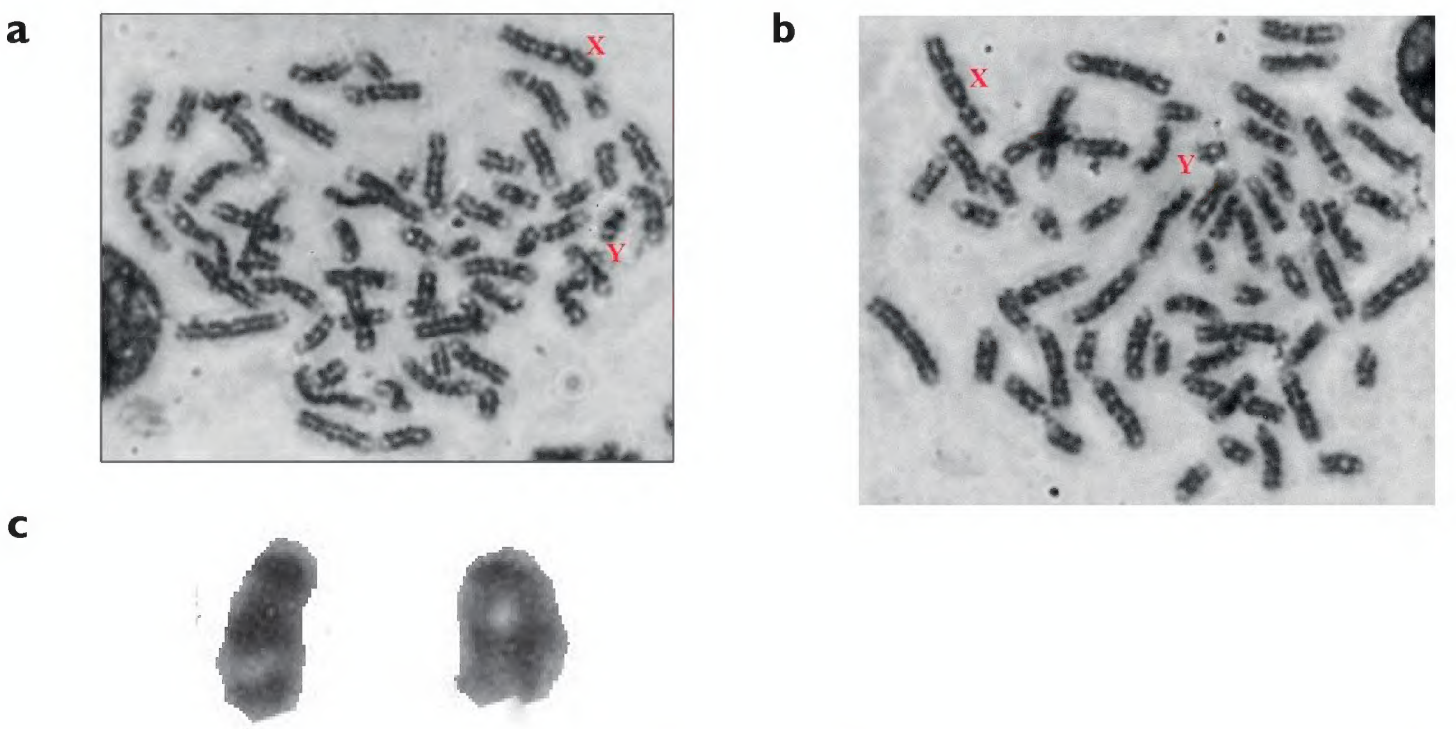

Figure 3. GTGbanded metaphase spreads of Kangayam bulls (**a**, **b**) with different Y-chromosome showing visible dark band in p-arm (**c,** left) and no prominent p-arm (**c**, right).

some and being situated together in the one arm (q) of acrocentric structure (Fig. 3), which is comparable to the standard G-banding pattern of *Bos indicus* cattle *viz.* Red Kandhari (Katkade 2005), Khillari (Nakod 2013), Malnad Gidda (Suresh et al. 2015), Indonesian native bulls (Ciptadi et al. 2017) and Nellore cattle (Amancio et al. 2019). The GTG-banding results also confirmed that there was no translocation between autosomes and Y-chromosome, as all the autosomes were having the typical G-banding pattern, as those of Iannuzzi and Di Meo (1995) and ISCNDB 2000 (Cribiu et al. 2001).

Of many studies pertaining to variations in morphology of cattle Y-chromosome, only a few cytogeneticists detected the polymorphisms. Halnan and Watson (1982) studied the Y-chromosome of *Bos indicus* breeds Sahiwal, Sindhi, Brahman, Santa Gertrudis and Belmont Red, derived from Zebu males, and reported as acrocentric though the centromere was found at a variable distance from the terminal point of the small p-arm; sometimes there would be no visible chromatin beyond the centromere and at other times the Y-chromosome would have distinct p-arms. Further, they also found visible subtelocentric Y-chromosome in every Sahiwal bull studied which they considered as differing only insignificantly from acrocentric according to the definition of Potter and Upton (1979) or Potter et al. (1979). According to them, these points served to raise the question of possible Y-chromosome polymorphism in *Bos indicus* in contradiction to the relative stability of the Y-chromosome in *Bos taurus*. Iannuzzi and Di Meo (1995) noticed that even though, there are size and morphological differences in the sex chromosomes (X and Y), R-banding patterns are conserved among cattle, river buffalo, sheep and goat. The differences are due to peri/paracentric inversions in sex chromosomes with loss or gain of heterochromatin.

In the present case also, there is a distinct p-arm in Y-chromosome of a Kangayam bull, when other bulls were possessing acrocentric Y-chromosomes with no prominent p-arm. As for the criteria for chromosome classification, the terms 'acrocentric' and 'subtelocentric' belong to different nomenclature systems, and their common use in the description of the same karyotype should be incorrect. Subtelocentric means the presence of telocentric, that is the chromosomes being strictly one-armed. It has been known from classic cytogenetics that the centromere is always distanced, at least minimally (very short arm) from the side opposite to the main (long) arm. Because of some uncertainty in definition of arm proportions in small chromosomes, such as the Y-chromosome of many mammals, including bovid taxa, and to stress a disproportion in arms of Y-chromosomes of the bulls studied, the term "subacrocentric" for the Y variant with a visible p-arm should be appropriate in recognition to the typical acrocentric of other breeding sires.

This visible p-arm appears attributable to heterochromatin variation and this can be considered as a possible Y-chromosomal polymorphism in Kangayam bull of south India. Even though, it is not a chromosomal abnormality, the productive and reproductive performance of the bull and its descendents should be studied to know the effect of the subacrocentric Y-chromosome; if the bull is allowed to breed without being usually culled upon receipt of the screening results.

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